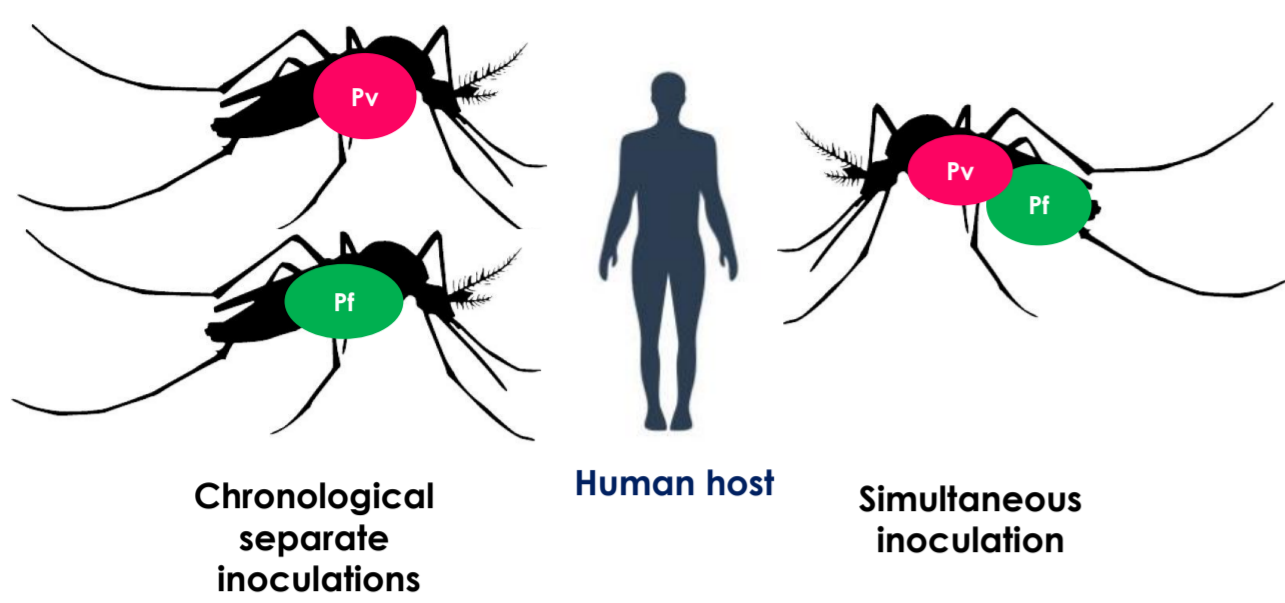


Clinical epidemiology and biology of mixed *P. falciparum* and *P. vivax* malaria in central India

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Mixed *Plasmodium* infections may occur either from:



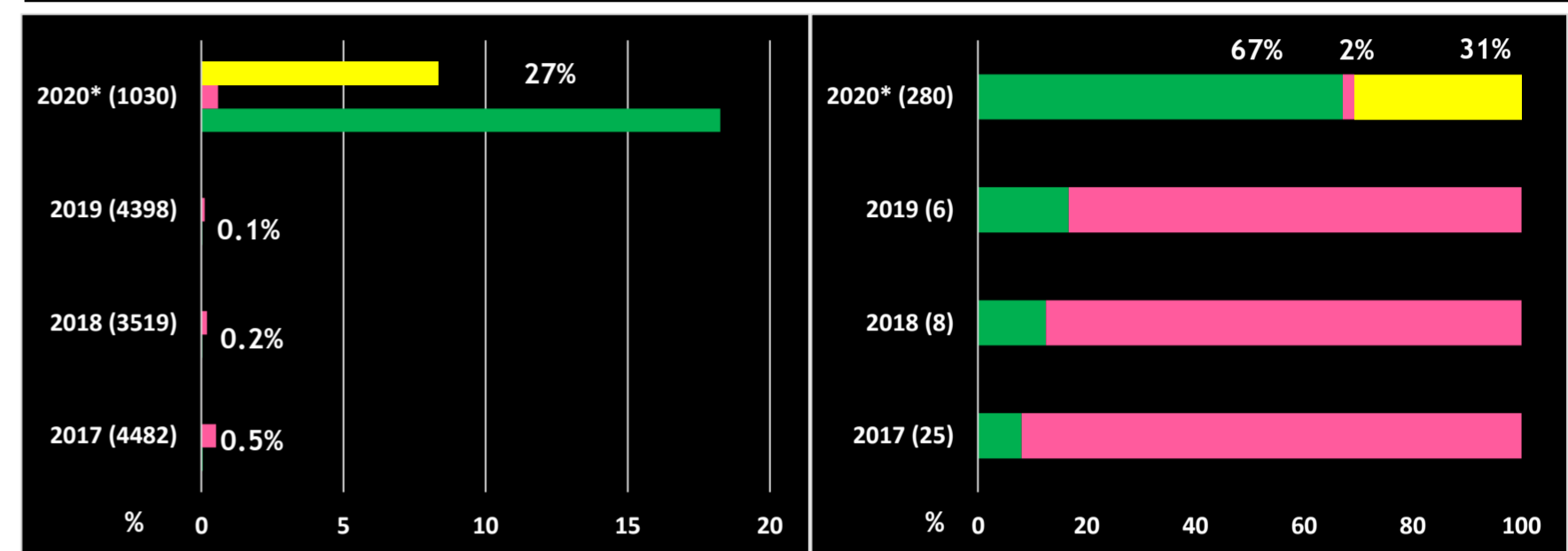
- Mixed infections could involve any combination of *Plasmodium* species, *P. falciparum* (Pf) & *P. vivax* (Pv) mixed infections demand greater attention
- Burden of malaria due to mixed infections is underreported (not reported under govt malaria surveillance programs)
- Prevalence reported by individual research studies: 4-46%
- No study reported to focus on its biology & implications
- It is unclear how individual species behave & interact with other species during mixed infections
- Co-existence of >1 species provides ample opportunities to sexually interact inside the mosquito (already reported in rodent parasites)

Objectives

- To estimate the prevalence & study the clinical profile and complications of *P. falciparum*-*vivax* mixed infections
- To develop a method to separate each species from mixed *P. falciparum*-*vivax* culture/infection
- To investigate hetero-species interactions (fertilization) between *P. falciparum* and *P. vivax* gametes

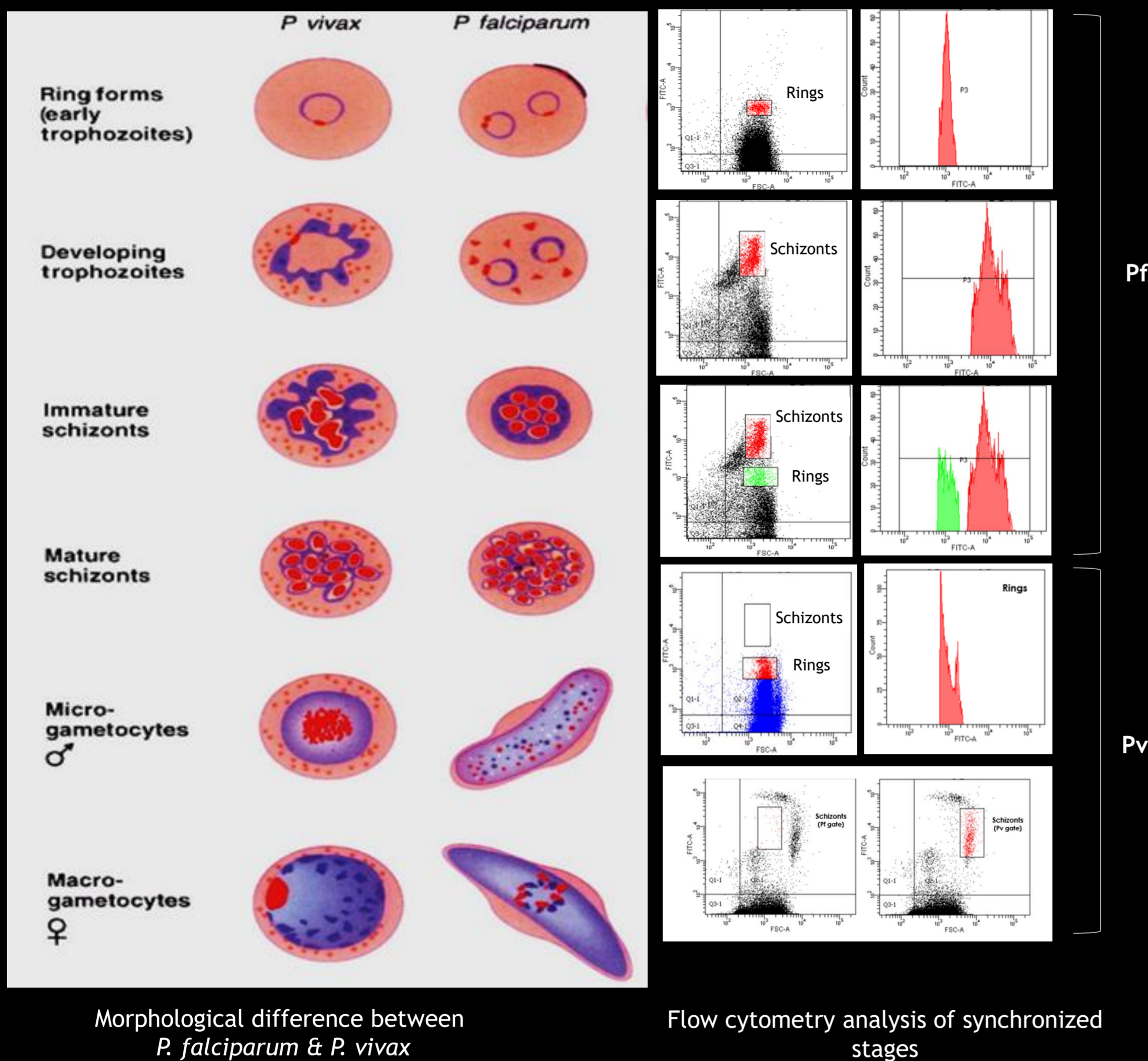
Prevalence of *P. falciparum* & *P. vivax* mixed infections

- A total of 1030 blood samples collected from June-Nov 2020 from 4 different government hospitals in central India
- Samples were screened by microscopy, RDT & PCR
- All patients with mixed PfPv infection followed telephonically till 12 months to identify associated complications & recurrences, if any



- Pf** Years 2017-2019: Data retrieved from the government surveillance program (Microscopy)
- Pv** Year 2020*: Current study outcomes (PCR)
- PfPv**
 - Light microscopy & RDT fail to detect all 27% (280/1030) mono & mixed infections
 - Current study reported 18% (188/1030) mixed PfPv infections in the year 2020
 - Zero mixed cases reported by government surveillance program in 2017-2019
 - During follow up, no clinical complications or recurrence observed

Separation of each species from mixed *P. falciparum*-*vivax* infections



- Different blood stages of both *P. falciparum* & *P. vivax* were synchronized with sorbitol (early stages) and MACS (late stages)
- Flowcytometry analysis of synchronized population was done to make stage-specific gates
- P. vivax* stages were analyzed on the same gates made for *P. falciparum* in order to see the difference

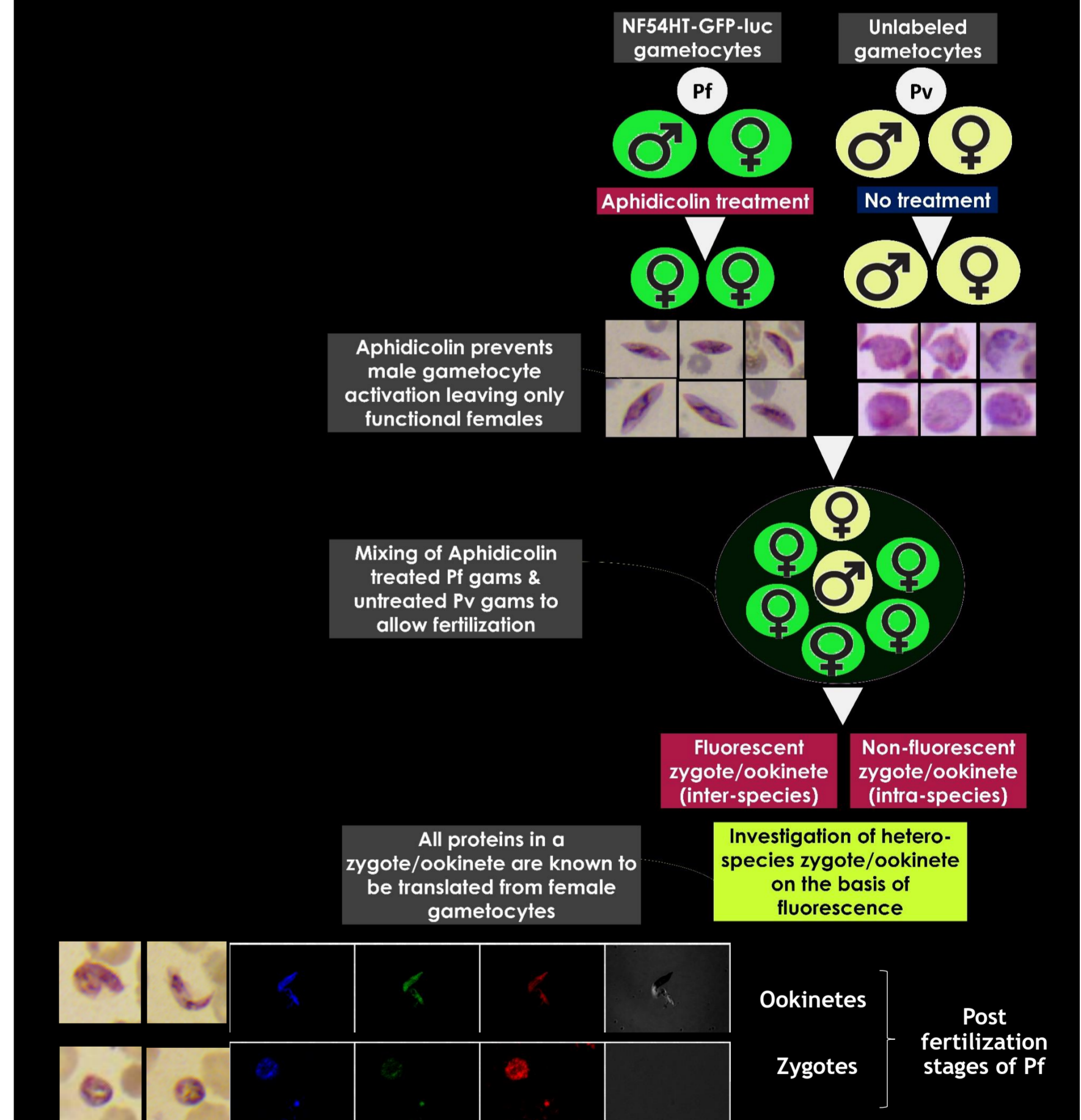
Outcome

P. falciparum & *P. vivax* were separable at the schizont-stage from mixed infections

Implications

The developed method would allow us to separate Pf & Pv from mixed infections in order to investigate parasite biology and infection dynamics of each species during mixed infections in terms of growth, development, response to antimalarials etc.

Investigation of interspecies fertilization between Pf & Pv



Outcome

- A platform was established to investigate fertilization between *P. falciparum* & *P. vivax*
- No zygotes/ookinetes observed in *P. vivax* (control; untreated) as well as in mixture of *P. falciparum* (females) & *P. vivax* (both male & female)

Major Challenge

- Lack of *P. vivax* *in vitro* culture
- Clinical isolates are unable to complete even one asexual cycle outside the host

Implications

Interspecies sexual interactions have the potential to influence the dynamics of malaria transmission & the established method could be further improved to investigate the same